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Ecophysiological adaptations of litter-dwelling Collembola and tipulid larvae

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Zusammenfassung : Ökophysiologische Anpassungen laubstreubewohnender Collembolen und Tipulidenlarven

Die spezifischen Lebensbedingungen in den verschiedenen Horizonten mitteleuropäischer Laubwaldböden haben zur Ausbildung charakteristischer physiologischer Anpassungen geführt :

1. Kleine edaphische Collembolenarten weisen einen vergleichsweise höheren Sauerstoffverbrauch auf als große Oberflächenbewohner. Kleine tiefenbewohnende Arten sind gegen Sauerstoffmangel weniger empfindlich.

2. In nasser Laubstreu lebende Larven von *Tipula maxima* ertragen Überflutungen wesentlich länger als Larven der Art *T. rubripes* aus trockeneren Waldbiotopen. Die Sauerstoffaufnahme von *T. maxima* ändert sich nach Überführung von Luft in Wasser nicht signifikant, während der O_2-Verbrauch von *T. rubripes* nach Überflutung stark abfällt. Der Sauerstoffverbrauch beider Arten ist von der O_2-Konzentration abhängig. Das Verhältnis von Sauerstoffaufnahme, Sauerstoffbedarf und verfügbarer Atemgasmenge wird mit sinkenden Wassertemperaturen günstiger.

3. Die Transpirationsrate edaphischer Collembolenarten in 84 % rel. Luftfeuchte ist wesentlich ausgeprägter als diejenige von Arten mit atmobiontischer Lebensweise.

4. Der Anteil hochungesättigter Fettsäuren in den Phospholipiden überwinternder symphypleoner Collembolen ist mehr als doppelt so hoch wie derjenige in nahe verwandten, ausschließlich sommeraktiven Arten.

5. Die Spektren kohlenhydratspaltender Enzyme im Darmtrakt ernährungsbiologisch repräsentativer Arthropodengruppen zeigen, daß die meisten Arten pflanzliche Zellwandbestandteile (Cellulose und ihre Abbauprodukte) nicht zu spalten vermögen.

Key words : Collembola, tipulid larvae, oxygen consumption, carbohydrases, ecophysiological adaptations.

I. Introduction

Litter inhabiting arthropods show characteristic features which are related to specific conditions of their habitat. (1) Small holes and cavernes in the litter put a limitation on body size and length of appendixes. (2) Dim light causes the reduction in the size of the eyes and amount of body pigmentation. (3) The high relative humidity leads to the great majority of litter-dwelling animals having a low resistance to desiccation. (4) The concentrations of oxygen and carbondioxid in the soil air and atmosphere above differ due to decomposition processes, respiration, restricted flow of air and soil flooding. (5) Structural plant polysaccharides like cellulose and hemicellulose are predominant litter components.

These special conditions of the litter environment have led to morphological, reproductive and physiological adaptations in the animals. Morphological and behavioral adaptations to these environmental factors have been known for many years (KÜHNELT, 1961; SCHALLER, 1964), whereas only a few investigations relate to ecophysiological adaptations with respect to respiration, transpiration and digestion. Significantly, the distribution of many litter inhabiting arthropods extend over a range of habitats and it therefore seems to be impossible to demonstrate the influence of all environmental factors acting within the litter with only one species. Therefore, the aim of our work was to compare different « life forms » (Lebensformtypen) by studying species of Collembola, tipulid larvae, oribatid mites, millipeds and woodlice which are among the most abundant and widespread of litter arthropods.

II. Influence of the pore-space system

Among litter inhabiting Collembola a distinction can be made between forms which live in the larger cavities of the top layer and those which live down in the pore-space system of the humus region. In general, these « life forms » (BOCKEMÜHL, 1956) have different body sizes and consequently different metabolic rates. Thus, the oxygen consumption of small edaphic species is significantly higher than that of large, surface inhabiting species (ZINKLER, 1966). Although this relationship was established for only one group of the Collembola (the Arthropleona) it also applies to a wide range of other litter-dwelling animals and is a reflection of the more general « surface law » of poikilothermes (PROSSER, 1973).

The influence of the pore-space system is indirect. The possession of a high metabolic rate (given as ml O_2 per g x h) may even be a disadvantage for small edaphic animals, but the total metabolism (given as ml O_2 per animal) of small species is lower than for larger species. This fact may be the reason why small animals held in 1 % oxygen show prolonged survival times (ZINKLER, 1966). Therefore, small species are more resistant to soil flooding.

III. Influence of low oxygen concentration

Complete flooding of the litter layers can occur. Some litter inhabiting species are brought to the surface by flooding whereas others remain underwater and most are killed within a few hours. Only well adapted species can persist in air bubbles, air filled pores or even in the water itself. Whatever the case, the available oxygen content of the water decreases rapidly with time. Tipulid larvae which are of great importance in moist habitats (BRAUNS, 1954), are particularly suitable for investigations of the effects of low oxygen concentration and to this end larvae of *T. rubripes* which prefer moist litter and *T. maxima* which live in extremely wet litter were studied. Preliminary experiments showed that the survival time of *T. maxima* larvae in water saturated with air at 18° C was more than 14 days, whereas the species from the dryer woodland community survived only one day. The survival time of *T. rubripes* is close to that of tipulid larvae from grassland (MEATS, 1970).

The different susceptibility of tipulid larvae to soil flooding may be due to a differing capacity for anaerobiosis or osmoregulation, different respiratory requirements, or differences in the oxygen content and temperature of the water, or combinations of these factors. Deoxygenated water is a lethal condition and leads to the death of both tipulid species within less than two hours. Survival times are not prolonged in water which is isoosmotic to tipulid body fluid. Thus, it appears, that anaerobiotic mechanisms and osmoregulatory activity are not responsible for the observed difference in susceptibility.

These differences are however related to metabolic rates. This was shown by measuring the oxygen consumption of both species in air (with a Warburg apparatus), during flooding with water saturated with air (using a Clark-electrode) and after returning the animals to air (Fig. 1). The result was that the oxygen consumption of *T. maxima* larvae did not change significantly during flooding. However, oxygen uptake of *T. rubripes* larvae decreased considerably during flooding and, during the first hour after being returned to air showed a significant increase, indicating an oxygen debt.

Larvae kept in water saturated with air live as if in running water, although stagnant water conditions are more typical in the natural forest litter. Therefore, the oxygen uptake of larvae was measured under the condition of a decreasing oxygen concentration (Fig. 2). It was found that for both species the oxygen consumption at 18° C decreases as the oxygen concentration in the water decreases.

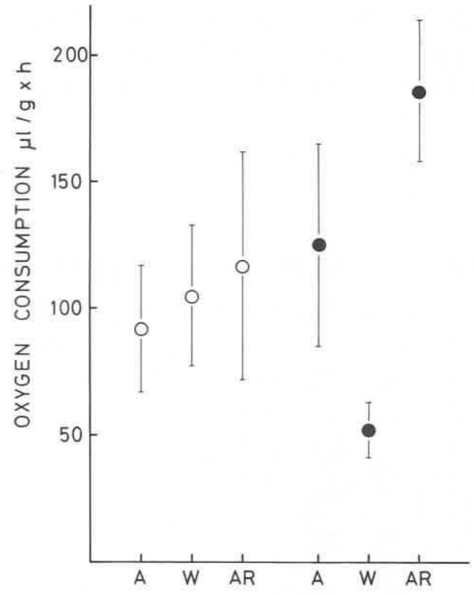

Figure 1 Rate of oxygen consumption of *T. maxima* (o) and *T. rubripes* (•) larvae in air (A), during flooding (3 hours) with water (W) and return to air (AR) at 18° C.

The survival time of submerged tipulid larvae increases as the temperature decreases (SCHAERFFENBERG, 1943; MEATS, 1970; ZINKLER, 1980), perhaps due to changes of the respiration rate. This possibility was tested by measuring oxygen consumption also at 4° C. It could be demonstrated that at low temperature the relationship between respiration rate, oxygen requirement and the availibility of oxygen is such that survival may be prolonged (Fig. 2). These conditions are far from optimal for the survival of *T. rubripes,* whereas the strong correlation between oxygen uptake and oxygen concentration does not hold for *T. maxima,* indicating a true resistance against flooding at low temperature.

IV. Influence of soil moisture content

The presence of capillary water ensures that the decomposing litter atmosphere is nearly saturated with water vapour whereas within the zone of freshly fallen litter the relative humidity may vary considerably. Different species of Collembola have a different resistance to desiccation according to whether they live in the epigeon or euedaphon. Numerous studies deal with the relationship between transpiration and the habitat (NOBLE-NESBITT, 1963; MAIS, 1970; VANNIER & THIBAUD 1978;

THIBAUD & VANNIER, 1980). The influence of the litter moisture content on the spatial distribution, transition to a dryer biotope and reproductive behavior of Collembola is also well established (VERHOEF & NAGELKERKE, 1977; BETSCH & VANNIER, 1977).

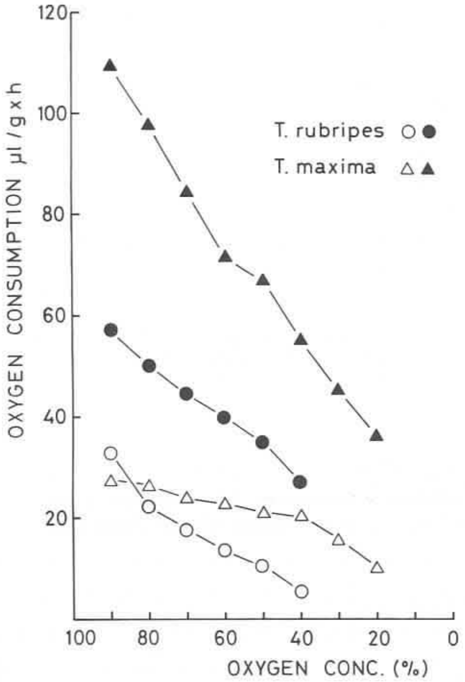

Figure 2 Rate of oxygen consumption of submerged *T. rubripes* and *T. maxima* larvae as a function of oxygen concentration of water at 18° C (closed circles, triangles) and 4° C (open symbols).

In our preliminary experiments, it could be demonstrated that Collembola are differently susceptible to even a slight lowering of the relative humidity. Thus, the loss of body weight of three species of Collembola was recorded with an electromagnetic balance (sensitivity 0.1 μg) at 18° C and 84 % relative humidity (Fig. 3). Within the weighing chamber the relative humidity was maintained with a KCl-solution and monitored with a hair hygrometer. The rate of water (weight) loss in the soil-dwelling *Onychiurus* was notably greater than for the surface living species *Allacma fusca* and *Tomocerus longicornus*. However, these findings do not imply that resistance against water loss enables species to go from moist litter layers to dry terrestrial places since a juvenile phase of *A. fusca* is very sensitive to desiccation (BETSCH & VANNIER, 1977).

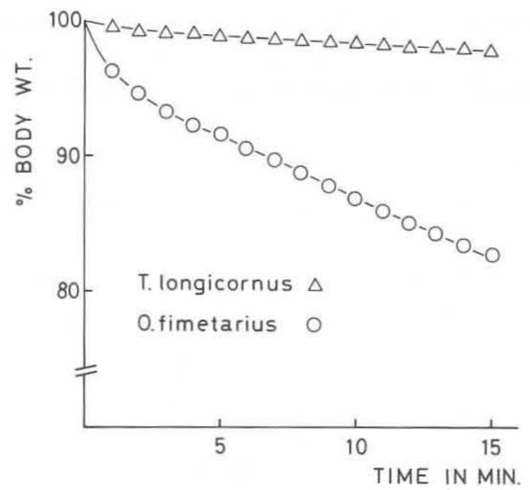

Figure 3 Water loss (% body weight) from the surface form *T. longicornus* and the soil-dwelling *O. fimetarius* as a function of time (84 % rel. humidity, 18° C).

V. The influence of low temperature

The temperature in litter environment shows less diurnal and seasonal variation than the air above. Nevertheless, during cold periods a number of overwintering species disappear from the top litter layers whereas other species survive as eggs or pupae. On the other hand, Collembola are little affected by low temperatures and can display their full range of activity even at freezing point. This adaptation may have its origin in the different fatty acid composition of membranes from overwintering forms (*Dicyrtoma fusca, Sminthurus aureus*) and summer active forms (*Allacma fusca*). Therefore, the fatty acid composition of phospholipids from whole animals was determined by thin layer chromatography and gaschromatography (methods described by ZINKLER, 1975). Linoleic, oleic and palmitic acids are the predominant fatty acids of phospholipid preparations in all three species. Arachidonic and eicosapentanoic acids are found in large quantities in *D. fusca* (16.3 %) and *S. aureus* (20.5 %) but not in *A. fusca* (8.3 %). Thus, the amount of long-chain polyunsaturated acids was significantly higher in overwintering Symphypleona, and this supports the view, that their membranes are more fluid than those of summer active species.

VI. The influence of litter food

A characteristic of litter is the large amount of hemicellulose, cellulose and other structural plant polysaccharides which come from dead leaves and decaying wood. These substances are potentially a rich source of energy. A large number of arthropods

living in the litter ingest such polysaccharides but it is not known whether or not they use them as an energy source. To answer this question the spectrum of carbohydrases present in various litter inhabiting animals was investigated.

The whole digestive tract of the following animals was prepared : the woodlouse, the pill millipede, larvae of *Tipula maxima*, the silverfish and a large Collembola *Tomocerus longicornus.* Oribatid mites and edaphic Collembolans were homogenized whole, due to their small size and/or thick cuticula. The methods for measuring carbohydrase activity are described by ZINKLER (1971, 1972).

All the species studied possessed a wide range of carbohydrases which hydrolized di- and polysaccharides. The most effective substrates were those with α-glucosidic linkages such as maltose, sucrose and starch. The possession of a ß-glucosidase, which hydrolizes cellobiose, is of special importance (Table I). Its natural function is usually assumed to be associated with the last step in the decomposition of litter cellulose. Large amounts of cellobiose is split only by silverfish and some oribatids (macrophytic and omnivorus feeders). The silverfish posseses a well characterized cellobiase and cellulase (LASKER & GIESE, 1956) and these were used as controls for the measurements of carbohydrase activity made here.

TABLE I

Hydrolysis of ß-glucosidic linked substrates by litter arthropods (in μmol glucose/g fresh weight x h).

	Cellobiose	CMC	Cellulose
Lepisma saccharina	450	43	5
Euzetes seminulum	1800	170	50
Pseudotritia duplicata	250	57	2
Damaeus auritus	22	-	-
Tomocerus vulgaris	-	-	-
Onychiurus fimetarius	21	-	-
Glomeris marginata	20	1	-
Porcellio scaber	5	<1	-
Tipula maxima	-	-	-

The other representatives of litter inhabiting animals examined showed little or no ability to attack ß-glucosidic linkages. Some oribatids and silverfish can also hydrolyze CMC, the carboxymethylderivative of cellulose, and to a much lesser extent, cellulose itself. The digestion of holocellulose by *Glomeris marginata*, as described by BOCOCK (1963), could not be confirmed. The role of the fermentation chambers in the hind gut of tipulid larvae remains unclear and the question of whether the observed carbohydrase activity was due to enzymes of the gut flora or the animal itself also remains unsolved.

These experiments indicate that the majority of litter inhabiting arthropods are not able to enzymatically degrade structural plant polysaccharides, although most ingest such matter. Therefore, these animals resemble saprophages in that they mechanically break down and excrete the plant material which is then more readily digested by bacteria and fungi.

Acknowledgements

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Discussion

VERDIER, B. : Do you think that your results on tipulids characterize more the higher insects with tracheal respiration than the lower ones such as collembolans with skin respiration, since we know that collembolans are well adapted to low O_2-concentrations ?

ZINKLER, D. : The survival of tipulids and collembolans after flooding can not be related to the difference between tracheal and skin respiration. Oxygen uptake of submerged tipulids is effected by skin respiration; this result was obtained by eliminating the tracheal system (GHILAROV & SEMENOVA, 1957; ZINKLER, 1980). The well-known adaptation of Collembola to low O_2-concentrations is not astonishing. In the air, oxygen content is about forty times higher than in the water. After flooding collembolans persist in air bubbles whereas tipulids remain in the water itself.

BLOCK, W. : Were you able to detect seasonal changes in the degree of unsaturation of phospholipid fatty acids within a Collembola species, or were your differences just between species ?

ZINKLER, D. : The degree of unsaturation of phospholipid fatty acids of *Tomocerus vulgaris* shows seasonal fluctuations. In spring, unsaturation is significantly higher than in early autumn. The peaks of low degrees of unsaturation persist during the first cold nights in September/October. Therefore, changes in the degree of fatty acid saturation can not be the sole explanation, especially for rapid temperature adaptations.

SWIFT, M.J. : You have shown the absence of the enzymatic capacity to break down plant cell wall components by most of the detritivores that you studied. Could you comment on their ability to digest fungal mycelium ? Do they, for instance, possess chitinases ?

ZINKLER, D. : Collembola possess chewing or sucking mouth-parts (WOLTER, 1963) that mechanically break down fungal hyphae. Few results indicate an enzymatic capacity to break down fungal mycelium cell walls : crude chitin (from crustaceans) is hydrolyzed to some extent by midgut homogenates of *Tomocerus longicornis*.

SZABÓ, I.M. : It seems to be difficult to detect the enzymatic adaptation of larger litter-feeding invertebrates to their food materials without the elimination of their gut-bacterial communities, the members of which can also produce enzymes in the digestive tract. Don't you think that gnotobiotic soil invertebrates would be the best subjects of such experiments ?

ZINKLER, D. : Gnotobiotic millipedes would be suitable to elucidate the role of their hindgut (fermentation chamber ?). Up to the present, the metabolic pathways of assimilation of leaf fibre by *Glomeris marginata* (ANDERSON & BIGNELL, 1982) remain unclear.